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By STIG LARSSON

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FROM THE DEPARTMENT OF PHYSIOLOGY, KUNGL. VETERINÄRHÖGSKOLAN AND
THE INSTITUTE OF RADIOPHYSICS, KAROLINSKA SJUKHUSET, STOCKHOLM

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To my Parents

Contents.

Preface.

The present investigation was carried out at the Department of Physiology, Kungl. Veterinärhögskolan and partly at the Institute of Radiophysics, Karolinska Sjukhuset, Stockholm.

To my chief and teacher, Professor YNGVE ZOTTERMAN, who directed my interest to these problems, I wish to express my warmest thanks for the interest he has shown in my work and for his valuable advice and criticism.

Some of the investigations were carried out in collaboration with Laborator ARNE FORSSBERG, Fil. D., at the Institute of Radiophysics, Karolinska Sjukhuset. I should like to acknowledge my great indebtness for the stimulating collaboration and for the interest he has shown in my work.

Laborator BENGT ANDERSSON, I want to thank for the great interest, good advice and valuable criticism he has shown in my work.

Professor AXEL PALMGREN has always been prepared to place his great experience at my disposal in the histological examination and I wish to express my gratitude to him.

Professor SVEN HOFLUND, I wish to thank for his valuable advice and helpfulness in questions concerning ruminant physiology.

For valuable help during the course of the investigation my sincere thanks are due to the staff at the Department of Physiology.

My work has been financially facilitated by contributions from "Anslag till främjande av Medicinsk forskning vid Veterinärhögskolan".

Introduction.

The problem of the regulation of food intake has in spite of its great importance received less attention than many other physiological problems. Many factors concerning the regulation of food intake are known but as a whole one can state that very little is known about its mechanism under physiological conditions.

Before this century three theories, still of some current interest, were advanced to explain the sensation of hunger. One theory referred hunger to peripheral, another to central, and the third to a sensation of general origin.

At present the most common conception is that food intake is regulated from a "center" in the brain sensitive to a certain as yet unknown hunger condition of the blood.

Thus BROBECK et al. (1951) suggested a "feeding center" localised to the lateral parts of the anterior hypothalamus. BROBECK's experiments have reopened the problem of the nature of the normal regulation of food intake.

The experiments to be described here are divided into two main parts. The first part deals with electrical stimulation of certain parts of the brain stem and with intrahypothalamic injections to provoke an increase of food intake. By these experiments, performed on sheep and goats, the subcortical localisation of some structures responsible for increase of food intake was determined. From these experiments, however, it was evident that nothing could be said about what really happened in those brain parts regulating food intake during hunger and satiation. Furthermore very little biochemical work has been done to analyse different parts of the brain under different physiological conditions. The second part, therefore, deals with experiments on rats undertaken to study whether any metabolic changes or differences in the brain exist during hunger as compared with satiation. This part of the investigation was undertaken in collaboration with Laborator ARNE FORSSBERG, Institute of Radiophysics, Karolinska Sjukhuset, Stockholm.

Review of the literature.

Before this century three theories were advanced to explain the sensation of hunger.

One theory tried to explain hunger as a sensation of peripheral origin. Thus stimulation of afferent nerves in general, evoked by some changes in all tissues, or of a more strictly localised group of afferents, mainly in the stomach, should result in hunger. This theory was introduced by HALLER (1776) and ERASMUS DARWIN (1801). HALLER thought that the empty stomach, by contractions, stimulated "hunger nerves" in the mucosa of the stomach by a grinding action. DARWIN put forward a contrary hypothesis in considering the empty stomach to be atonic and referring the peripheral hunger feelings to absence of contractions. Yet both HALLER and DARWIN were of the same opinion in referring hunger to a peripheral origin.

It was later shown by CANNON and WASHBURNE (1912) and CARLSON (1916) that the subjective feeling of hunger pangs coincided with particular hunger contractions in the empty stomach. They stated that these were the eliciting moments for the sensation of hunger.

It was, however, shown especially by SHERRINGTON (1900) and GROSSMAN and STEIN (1948) that total denervation or operative removal of the stomach had no dominant influence on the regulation of food intake from this point of view. CARLSON et al. later suggested that hypoglycemic conditions could have some influence on the origin of hunger contractions (BULATAO and CARLSON, 1924). They also found that hunger contractions were inhibited by intravenous injections of glucose and that injections of insulin by its depressing action on blood glucose strengthened hunger contractions.

Later SHARE et al. (1952) uncovered two inhibitory mechanisms influencing intake of food. The first was initiated by gastric distention, and the second an extragastric factor of caloric origin.

Before these investigations MAGENDIE (1826), MILNE-EDWARDS (1878) and others, had advanced a theory in which they postulated

a hunger center in the brain, sensitive to a starvation state in the blood. According to MAGENDIE hunger was of central origin. He denied the occurrence of hunger contractions but did, however, partly accept the theory that sensory impulses from different organs could provoke hunger under certain circumstances. In opposition to the theories that thirst and hunger were of peripheral origin, DU BOIS-REYMOND (1910) stated, that the conceptions about dryness in the mouth and emptyness in the stomach as eliciting moments for the two sensations were wrong.

In recent years it has also been found that the sensation of thirst probably originates from structures situated in the hypothalamus (FISCHER, INGRAM and RANSON, 1938; HEINBECKER et al., 1944, and ANDERSSON, 1953).

DU BOIS-REYMOND distinguished between two kinds of hunger, namely vagal hunger and tissue hunger. His theories were mostly in accordance to the third main theory introduced by ROUX (1897 and 1907) and FOSTER (1891) who suggested that a hunger center in the brain would be stimulated both by a starvation state in the blood and by afferent impulses from all organs in the body. Thus this theory referred hunger to a sensation of general origin.

With the evolution of the experimental technic it was possible to attack the problem of the regulatory mechanism for food intake from other sides. HESS (1932) introduced a new technic for electrical stimulation of different parts of the brain in unanaesthetised animals. He made systematical electrical stimulations of the hypothalamus in cats and many problems were solved by these investigations, especially those concerning the central organisation of the vegetative nervous system. With the methods of HESS and of HORSLEY-CLARKE (CLARKE, 1939) it was also possible to make discrete lesions of different parts of the brain and study the insufficiency symptoms. BRÜGGER (1943) who compiled the results of electrical stimulations in the hypothalamus, earlier obtained by HESS, found that stimulation in the vicinity of the mamillo-thalamic tract resulted in bulimia. The animals got a pronounced urge to eat — not only food but even indigestible material.

Other hypothalamic structures, however, were also found to influence food intake. It was thus shown that bilateral destruction of the ventro-medial hypothalamic nucleus was followed

by the development of obesity (HETHERINGTON, 1941 and 1944; HETHERINGTON and RANSON, 1942; BROBECK, TEPPERMAN and LONG, 1943; and HEINBECKER et al., 1944). The obesity was found to be the result of a primary increase of food intake. ANAND and BROBECK (1951) found in rats that bilateral destruction of the lateral parts of the hypothalamus structures was followed by complete absence of eating. These areas included the extreme parts of the lateral hypothalamic nucleus at the same rostro-caudal level as the ventro-medial nucleus. ANAND and BROBECK introduced the theory of a "feeding center" situated in the hypothalamus, with its lateral part exerting the more basic type of the control of food intake. The activity of this part was postulated to be partly regulated by the medial component of the "feeding center" through lateral connections. DELGADO and ANAND (1953) could provoke an increase of food intake after electrical stimulation of the lateral hypothalamus at the same rostro-caudal level where BROBECK et al. got their effect. The animals did not eat during the stimulation proper, but their food intake was increased for some days afterwards. DELGADO and ANAND found no variations in blood sugar values during the course of the experiments. A suggestion was made that food intake was concerned with humoral factors.

The question of the eliciting moments for food intake was discussed by MAYER et al. and BRUCE and KENNEDY (1951) who made series of experiments to investigate this problem. They tried to put the findings by BROBECK et al. in relation to biochemical processes in the brain and other parts of the body. MAYER as a working hypothesis suggested a glucostatic mechanism responsible for the regulation of food intake. This hypothesis was based upon certain experimental findings. It was thus shown that substances taking part in the carbohydrate metabolism were probably the most active component in this mechanism. It was also found that variations of food intake were strikingly correlated to variations of blood glucose and were influenced by other metabolites causing direct or indirect variations of blood glucose.

Before the investigations of MAYER et al. the relation of the blood sugar level to the occurrence of hunger and hunger contractions was studied by GROSSMAN and STEIN (1948) and by JANOWITZ and IVY (1949). They found that hunger did not set in until the blood sugar had started to rise. JANOWITZ and IVY also showed that hunger occurred on the average 27 minutes after the lowest

blood sugar level. These investigations were in accordance with those found by SCOTT et al. (1938) who made systematic investigations in man of the blood sugar values and the periods of hunger. The blood sugar values did not change more than ± 5 mg % from the mean values and this was taken as evidence that the blood sugar level did not have any influence on the hunger periods in man under physiological conditions.

Old and new clinical observations have shown that injuries in certain parts of the brain often result in disturbances in the normal regulation of food intake. The clinical observations support the theory of the existence of structures in the brain regulating food intake. PAGET (1897) described several brain lesions in man followed by more or less pronounced bulimia. The pathological changes were mostly localised to the vicinity of the third ventricle in the hypothalamic region. Such observations have since been confirmed by several investigators (FUTER, 1940; KIRSCHBAUM 1951; and others).

The so called hypothalamic obesity was described by ERDHEIM (1904) and this finding was later taken as evidence that structures in the brain, probably in the hypothalamus, could induce an increase of food intake. Furthermore it was shown that hypothalamic obesity was largely unrelated to so called pituitary obesity (ASCHNER, 1912; SMITH, 1927; HETHERINGTON and RANSON, 1940).

Beside lesions of the hypothalamus it has also been shown that destruction of the connections between the thalamus and the frontal lobes may influence the regulation of food intake. Thus COBB (1944) showed that frontal lobotomy in man resulted in an increase of food intake in about 90 % of the cases of which about 60 % was permanent. In investigations, not yet published, DELGADO in monkeys has shown how moods as controlled by the frontal lobes of the brain have a tendency to regulate the desire for food.

Investigations have also been undertaken to study possible pathological changes in the brain of patients with diabetes mellitus. Thus MORGAN et al. (1937) found pathological changes especially localised in the cells of the paraventricular nucleus, which cells showed pronounced cromatolysis and neuronophagia. The authors suggested that the paraventricular nucleus had a differentiated ability to be stimulated by chemical changes in the blood by substances taking part in carbohydrate metabolism.

Biochemical investigations were undertaken to see if any characteristic changes in cerebral metabolism could be found during conditions of hunger. KERR and GHANTUS (1936) found that the amount of brain glycogen was not altered during starvation. This investigation was made on comparatively large samples of brain.

However, very little is known about differences in the metabolism of various parts of the brain stem, especially in different physiological conditions. BORELL and ÖRSTRÖM (1945) studied the incorporation of radioactive phosphorus (^{32}P) in different regions of the brain in rats and rabbits. They found that great differences existed in different parts of the brain. KELLER and ROBERTS (1953) reported that the glucose consumption of hypothalamic tissue in vitro was increased after administration of epinephrine.

WEIL-MALHERBE (1952) stated that when studying the metabolic processes of the brain the method of killing the experimental animals is very important. Thus it was shown that decapitation will not stop the biochemical processes and results obtained by this method of killing are therefore difficult to interprete.

PART I.

Hyperphagia from stimulation of the hypothalamus and medulla in sheep and goats.

It was previously shown by BRÜGGER (1943) that electrical stimulation of certain parts of the hypothalamus in the cat produced an increase of food intake. Other findings (BROBECK et al. and others), however, suggested the existence of special structures responsible for the regulation of food intake in other parts of the hypothalamus *viz.* in the anterio-lateral part.

The present investigations were undertaken to see if any correlation could be found between the findings of BRÜGGER and of BROBECK et al. For that reason electrical stimulation of the hypothalamus in sheep and goats was made. In other experiments the medulla oblongata was stimulated. Further, intrahypothalamic injections were made in goat. Particular attention was paid to the increase of food intake and to some other autonomic functions *e. g.* rumination. An attempt to correlate the effect on food intake to masticatory and licking movements was also made.

A. Electrical stimulation of the hypothalamus and medulla.

ANDERSSON (1951) introduced Hess' technic of electrical stimulation of the brain stem in sheep and goats, and especially the goats proved to be well suited for experiments of this kind.

Methods:

The experiments were performed in sheep and goats of both sexes.

Preparations of the animals before the experiments: The animals received food ad libitum in the morning the same day as the experiments were performed. This was done to ensure that greater food intakes during the experiments were due to the stimulations. To enable a more objective evaluation of the experiments the animals were filmed before, during and after the stimulations.

Electrical stimulation: Hess' technic of electrical stimulation in unanaesthetised animals was used, with slight modifications, partly described by ANDERSSON (1951).

The skulls of the animals were roentgened before the experiments to determine the right position for the electrodes. In sheep and goats the size and the form of the skulls vary so much that this procedure was necessary. In order to facilitate the measurements of the skulls after roentgening steel needles were placed in the frontal or parietal bones of the animals (Fig. 1). The operations were performed under local anaesthesia (Xylocain-Exadrin, Astra). The animals were placed in a special operation stand and were as a rule very calm during the operations.

A metal socle (Fig. 2, *1*) was fixed on the skull by screws (*2*). To make the holes in the skull a burr-drill was used. Through the holes on each side three parallel electrodes (*6*) joined by an insulating base (*5*) were put into the brain. To facilitate correct

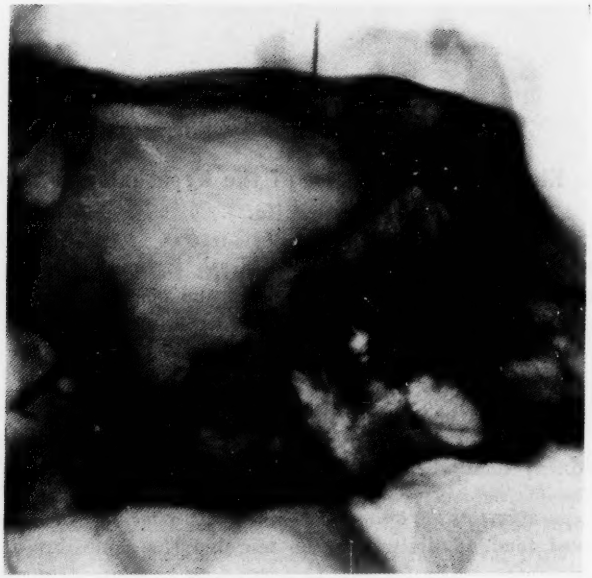

Fig. 1. *Roentgen picture of the skull of a goat before operation.* Note the steel needle.

insertion of the electrodes two removable guides (*4*) were attached to smaller guides (*3*) fixed at the socle. The electrodes were insulated except at their points (*7*) where the stimulus current could be transferred into the surrounding tissue. Before the electrodes were placed into the brain, holes were made in the meninges with a puncturer (*13*). Unipolar stimulation was used. The cathode was connected to the electrodes by insulated wires (*9* and *11*) and the socle was used as an anodal indifferent electrode (*10*). The electrodes could be inserted at different levels by putting pieces of brass of different thickness between the socle and the electrode connection (*8*). In this way it was possible to get several points of stimulation. The electrodes were connected by thin wires (*9*) to an insulated plate (*12*) fixed on the socle. From these connections other wires (*11*) fixed to a balanced suspender in the roof were running. This arrangement was made to avoid tension on the wires between the stimulation box and the electrodes.

In all the experiments the animals could move freely in a pen.

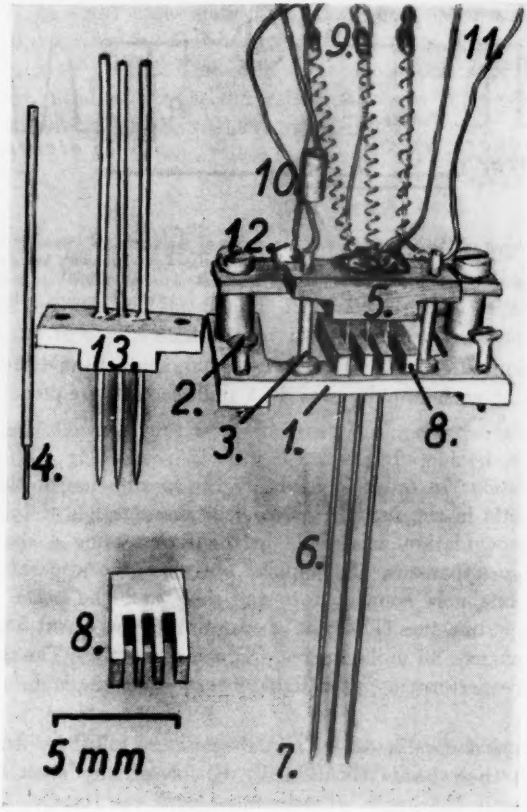

Fig. 2. *The electrode arrangement and materials used for electrical stimulation.* *1.* Socle, *2.* Screw to fix the socle, *3.* Fixed electrode guide, *4.* Removable guide, *5.* Insulating base, *6.* Electrodes, *7.* Uninsulated points of the electrodes, *8.* Pieces of brass to obtain different levels for stimulation, *9.* Insulated electrode-connections, *10.* Anodal indifferent electrode, *11.* Insulated wires from the apparatus used for stimulation, *12.* Insulated plate, *13.* Puncturer.

The stimulating current: The type of pulsating current was that recommended by HESS. From an electronic generator the original square wave (Fig. 3) passed through a band-pass filter (*A*) consisting of the internal resistance (R_i) of the square wave generator and two condensers (C_1 and C_2). The filter dampened the edges of the impulses and also removed the D. C.-component to avoid

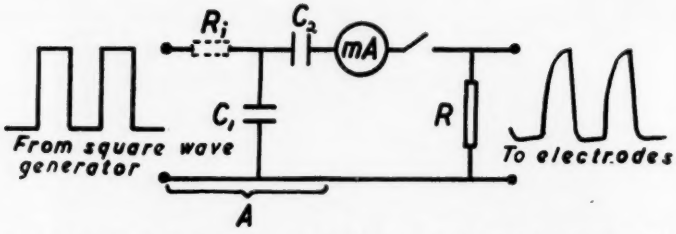

Fig. 3. *Scheme of the apparatus and the shape of the electrical impulses used for stimulation.* A = Band-pass filter consisting of: R_i = Internal resistance of the generator; C_1 and C_2 = Condensers. R = Resistance allowing residuating polarisation to escape during the intervals between stimulus pulses.

polarisation effects at the electrodes during stimulation. The duration of each impulse was 12.5 milliseconds, frequency from 8 to 30 c/s.

Coagulation: In order to facilitate the localisation of the electrode points in the further histological examination of the brain a small coagulation was made in the tissue using a spark-gap diathermic apparatus. The bipolar outlet of the apparatus was used — one pole connected to the socle and the other to the electrode in question. The time of coagulation was about 5 seconds and the current 40 milliamperes, frequency 0.9 Mc/s. The coagulations were performed under light general anaesthesia.

Histological examination: The animals were killed by decapitation and their heads fixed in 10 % formaldehyde or Bouin's fluid. Mostly the heads were perfused with the fixative fluid to be used. The brains were embedded in paraffin and cut in serial sections, horizontal or transverse. The sections were alternatively stained with cresyl violet (B. D. H.) or toluidine blue (Gurr) and with the nerve staining method described by PALMGREN (1948) which for this purpose seemed to be superior to other nerve staining methods.

Determination of blood sugar: Blood samples were taken before, during and after the experiments to determine if any changes in the amounts of reducing substances could be found. The blood samples were drawn from the jugular vein. The analysis method described by FOLIN-WU (1920 and 1930) was used as a routine

method. In some cases determinations were also made by the methods described by HAGEDORN, HALSTRÖM and JENSEN (1935) and SOMOGYI (1945). The differences in amounts of reducing substance obtained by the different methods were in accordance with those found by KINGSLEY and REINHOLD (1949).

Duplicate samples were taken as a rule, and the mean value used.

Results:

Electrical stimulation of certain parts of the hypothalamus and the medulla in sheep and goats resulted in hyperphagia. In relation to this, attention was also paid to other effects such as rumination, licking and mastication.

In the following survey of the results some experiments are reported. In this causuistic the electrodes are named *A*, *B* and *C*; *A* being the most anterior electrode.

The following abbreviations are used:

Right side = *r*, left side = *l*.

Upper level of stimulation = *o*, middle level = *m* and lower level = *u*.

Voltage = *Str.*, Impulse frequency = *Fr.*, Duration of the stimulus = *Dur.*

These abbreviations are mostly the same as those used by HESS and ANDERSSON.

Figure 4 shows a paramedian sagittal section through the hypothalamus of a goat with the electrodes situated with their points at the lower level.

I. Electrical stimulation of the hypothalamus.

Experiment no. 2/53 (Lactating goat).

The stimulations were performed at three levels on the left side. The distance between the levels was 1 mm. The electrode *A. l. o.* (*Str.* 0.5—1 V., *Fr.* 20 imp./sec., *Dur.* 60 sec.) gave at stimulation hyperphagia mixed with rather pronounced masticatory and licking movements which did not vary with the frequency.[1] After stimulation the animal stopped eating but mas-

[1] In the following this kind of mastication and licking mixed with hyperphagia are called "extra" movements.

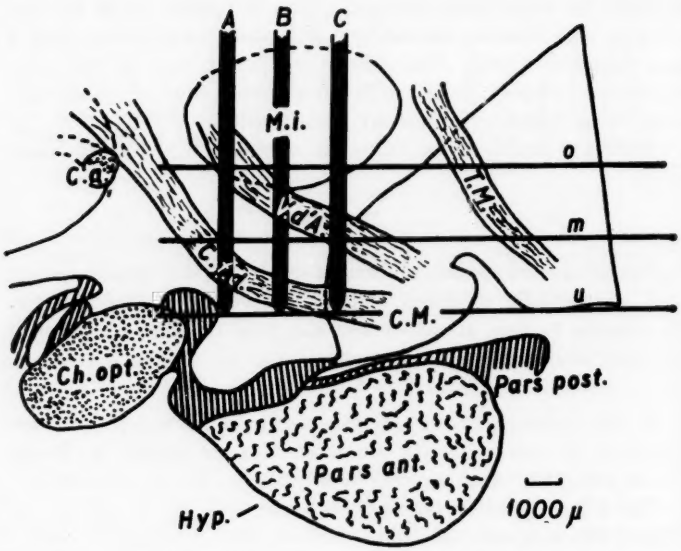

Fig. 4. *Paramedian sagittal section through the hypothalamus of a goat.* The electrodes are situated with their points at the lower level.

A, *B* and *C* = electrodes; *o*, *m* and *u* = Different levels of stimulation. *C. a.* = Anterior commissure, *C. j. d.* = Columna fornix descendens, *Ch. opt.* = Optic chiasma, *C. M.* = Mamillary body, *Hyp.* = Hypophysis, with *Pars ant.* and *Pars post.*, *M. i.* = Massa intermedia, *T. M.* = Tractus Meynert, *V. d'A.* = Mamillo-thalamic tract (Tractus Vicq d'Azyr).

tication and licking continued for a while. Stimulation at *A. l. m.* and *C. l. o.* gave, under the same stimulatory conditions, very pronounced masticatory and licking movements varying with the frequency.

Coagulation was undertaken at point *A. l. o.*

During the course of the experiment the animal was very calm and behaved normally. It was sacrificed after 12 days with clinical signs of anterior stenosis. The animal was autopsied at the Department of Pathology, Kungliga Veterinärhögskolan, and the clinical diagnosis verified.

Localisation: The points of the three electrodes at level *o* are projected in Fig. 6. The electrode *A. l. o.* was situated just lateral to the mamillo-thalamic tract.[1]

[1] In the figures over the localisation, the stimulation points in the horizontal plane, in some cases, vary ± 500 micra.

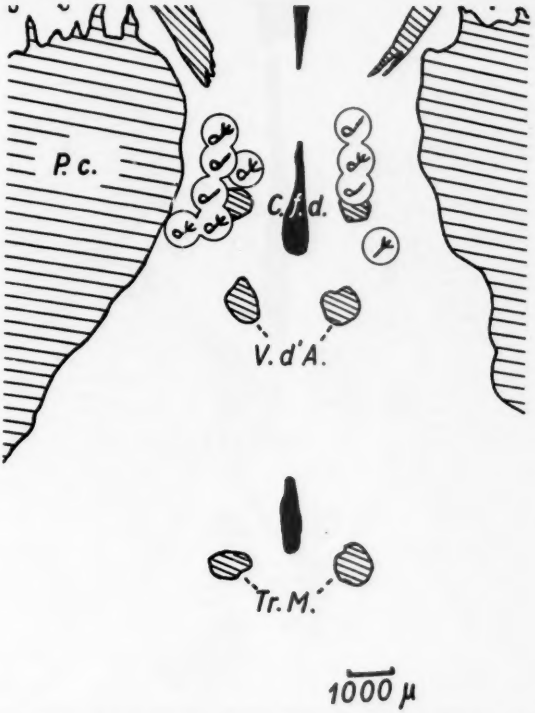

Fig. 5. *Diagram of a horizontal section between the levels o and m in Fig. 4.*
⊘ = Licking movements, ⊙ = Masticatory movements.
C. f. d. = Columna fornix descendens, *P. c.* = Cerebral peduncle, *Tr. M.* = Tractus Meynert, *V. d'A.* = Mamill-othalamic tract (Tractus Vicq d'Azyr).

The points where mastication and licking were obtained at level *m* are seen in Fig. 7.

Experiment no. 3/53 (Lactating goat).

The electrodes were inserted into the left side of the brain and stimulations were made at three levels. The distance between the levels was 3 mm. Stimulation at *A. l. m.* (*Str.* 0.5—1 V., *Fr.* 20 imp./sec., *Dur.* 60 sec.) resulted in pronounced hyperphagia together with "extra" masticatory and licking movements. During stimulation at *B. l. u.* the animal showed masticatory and licking movements and repeated ructus.

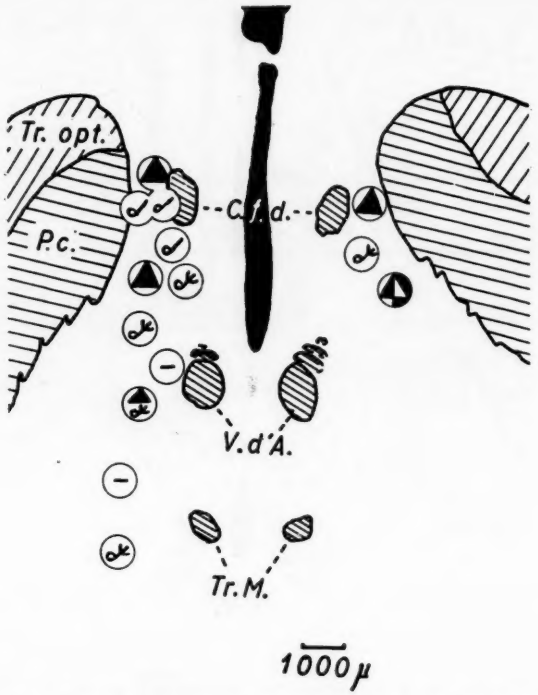

Fig. 6. *Diagram of a horizontal section just below level m in Fig. 4.*
▲ = Increase of food intake, ▲ = Rumination, ✓ = Licking movements, ✓ = Masticatory movements, ⊖ = Resultless points.
C. f. d. = Columna fornix descendens, *P. c.* = Cerebral peduncle, *Tr. M.* = Tractus Meynert, *Tr. opt.* = Tractus opticus, *V. d'A.* = Mamillo-thalamic tract (Tractus Vicq d'Azyr).

No significant variations in the blood sugar values were observed until after coagulation; then the blood sugar value rose about 30 mg %. The animal was very calm during the experiment.

Localisation: The points of the electrodes at the middle level are projected in Fig. 6. The electrode *A. l. m.* was situated just lateral to and between the columna fornix descendens and the mamillo-thalamic tract. *B. l. u.* (Fig. 7) was situated lateral to this tract.

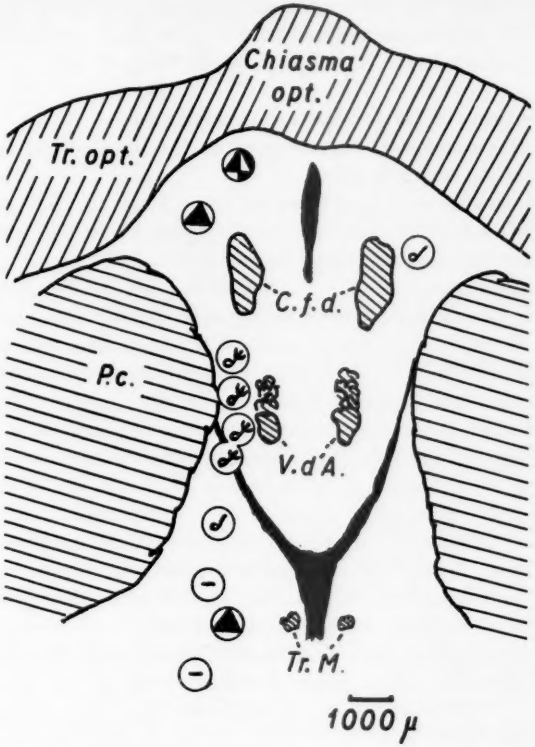

Fig. 7. *Diagram of a horizontal section at level u in Fig. 4.*
▲ = Increase of food intake, ◆ = Rumination, ✓ = Licking movements, ✓ = Masticatory movements. ⊖ = Resultless points.
The same abbreviations as in Figs. 5 and 6 are used.

Experiment no. 4/53 (Male goat, 6 months).

The electrodes were inserted into the left side of the brain. Two levels were used. Hyperphagia was obtained when *A. l. o.* was stimulated (*Str.* 0.5—1 V., *Fr.* 9 imp./sec., *Dur.* 70 sec.). *A. l. o.* was the only point stimulated where any visible effect was obtained.

The animal was sacrificed after about one month. On autopsy the rumen appeared to be moderately enlarged and the omasum diminished. Coagulation was performed at *A. l. o.*

Localisation: The electrodes at level *o* are projected in Fig. 7. The point of the electrode *A. l. o.* was situated caudo-lateral to the mamillary body, just lateral to the tract of Meynert.

Experiment no. 8/51 (Lactating sheep).

The electrodes were first inserted on the right side of the brain and then on the left side.

Stimulations were performed at three levels on each side. Immediately after the electrodes on the right side were inserted into the brain the animal began to ruminate.

On stimulating at *B. r. o.* (*Str.* 1 V., *Fr.* 30 imp./sec., *Dur.* 60 sec.) the animal showed pronounced licking movements and nosed its food. This effect could be repeated as soon as the stimulations set in. At *C. r. o.* (the same stimulatory conditions) the animal got frequent ructus more often than normal. On auscultation the rumen contractions were heard more distinct and frequent than before. Repeated stimulations at this point resulted in rumination. Stimulation at *A. r. o.* under the same stimulatory conditions resulted in hyperphagia. Stimulations at *A. r. u.* were resultless. On renewed stimulation at *C. r. o.* the animal showed hyperphagia together with "extra" masticatory and licking movements. On the left side stimulation at *B.* and *C. l. o.* resulted in licking movements.

No variations of blood sugar were obtained until after the experiment when the blood sugar increased from 67 to 101 mg %. During the experiment the animal was very calm. Coagulation was made at *C. r. o.*

Localisation: The electrodes at level *r. o.* are projected on Fig. 6. Point *A. r. o.* was situated just lateral to the columna fornix descendens. Point *C. l. o.* was situated antero-lateral to the columna fornix descendens (Fig. 5).

II. Electrical stimulation of the medulla.

Experiment no. 6/51 (Castrated ram).

The electrodes on both sides were used. Three stimulation levels (distance 2 mm) were used. Stimulation of the over and under levels resulted in motor movements. When the point *C. l. m.* was stimulated (*Str.* 0.5 V., *Fr.* 20 imp./sec., *Dur.* 60 sec.) the animal began to eat eagerly straw, even if contaminated with urine. Coagulation was made at this point of stimulation.

Fig. 8. *Diagram of a transverse section through the medulla at the level of the inferior olivary nucleus.*

▲ = Increase of food intake.

N. d. X. = Dorsal motor nucleus of vagus, *T. s.* = Tractus solitarius, *F. l. m.* = Medial longitudinal fasciculus, *L. m.* = Medial leminiscus, *O. i.* = Inferior olivary nucleus, *T. p.* = Corticospinal tract, *C. r.* = Corpus restiforme.

Localisation: The point *C. l. m.* (Fig. 8) was situated in the region of the dorsal motor nucleus of the vagus.

Experiment no. 5/53 (Lactating goat).

The electrodes were inserted into the right side and stimulations made at two levels (distance 2 mm). When the lower level was stimulated the animal showed motor movements of the right foreleg. Stimulation of *A. r. o.* resulted in hyperphagia (*Str.* 1 V., *Fr.* 20 imp./sec., *Dur.* 60 sec.). The latent period was about two seconds and the animal continued eating for about half a minute after stimulation. The effect could not be repeated until about 10 minutes after the former stimulation. Coagulation was undertaken at *A. r. o.*

Localisation: The site of the electrode *A. r. o.* is seen in Fig. 8. The localisation corresponded to the lateral region of the dorsal motor nucleus of the vagus.

A summary of the casuistics and Fig. 5—8 shows:

1. Hyperphagia was obtained at 8 points of stimulation of the hypothalamus and of the medulla at 2 points.

2. In the hypothalamus the localisation of the points showed a lateral distribution from just caudal to the optic chiasma backwards through the hypothalamus. The anterior points corresponded to the localisation of the lateral hypothalamic nucleus.
3. In two cases an increase of food intake was observed on stimulation of the region of the dorsal motor nucleus of the vagus.
4. In two cases rumination was obtained at points where the first stimulation resulted in hyperphagia.
5. Caudal to the columna fornix descendens the hyperphagia was often intermixed with "extra" masticatory and licking movements.
6. Masticatory and licking movements were obtained caudal to the columna fornix descendens in the middle and ventral parts of the hypothalamus and in the dorsal part close to the columna fornix descendens.

These results will be discussed together with those obtained by intrahypothalamic injections.

B. Intrahypothalamic injections in goats.

The experiments described above showed that hyperphagia, rumination and related motor effects such as mastication and licking could be obtained by electrical stimulation of the hypothalamus and the medulla in sheep and goats.

The experiments to be described here were undertaken to determine if any substances reacted specifically in provoking hyperphagia when introduced directly into the hypothalamic regions in question. A further object was to study the non-specific osmotic effect in the same structures.

Methods:

Goats of different ages and sexes were used for the experiments.

The preliminary preparation of the animals was the same as for the electrical stimulations. In most experiments the animals were placed in a pen where they could move freely, but in some experiments the animals were placed in a modified Pavlov-stand.

Before, during and after the experiments the skull of each animal was roentgened to determine the right position for the electrodes. As in the electrical stimulations the skulls of the animals were provided with steel needles before roentgening.

Injection technic and apparatus:

ANDERSSON and LARSSON designed a new apparatus for intracerebral injections of very small amounts of solutions. Figure 9 shows the injection apparatus used. The socle was almost the same as that used for the electrical stimulations (Fig. 9, *1*). The socle was fixed on the skull by screws. The burr holes were made smaller than before and to get them parallel a burr-guide (*2*) was fixed on the socle. This consisted of a piece of brass provided with parallel holes of determined diameter, corresponding

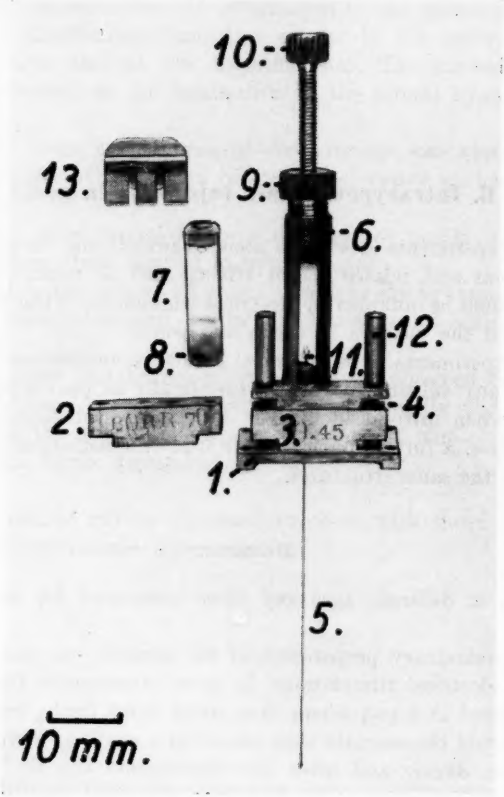

Fig. 9. *Injection apparatus.* *1.* Socle, *2.* Burr-guide, *3.* Cannula-guide, *4.* Screws fixing the cannula-guide, *5.* Cannula, *6.* Mantle, *7.* Glass ampulla, *8.* Rubber stopper, *9.* Screw holding the ampulla, *10.* Screw to inject the solutions, *11.* Upper part of the cannula, *12.* Screw to fix the injecting apparatus, *13.* Pieces of brass to obtain different stimulation levels.

to the external diameter of the burrs. The burr-guide was removed and a cannula-guide (*3*) was fixed on the socle by screws (*4*). The holes of the cannula-guide were of the same diameter as the cannula (0.35—0.45 mm). The injecting apparatus consisted of a mantle (*6*) fixing a glass ampulla (*7*) closed with rubber (*8*) at the ends. Two screws were attached at the top of the mantle, one (*9*) fixing the ampulla in the mantle and the other (*10*) used

to press the solutions in the ampulla into the cannula. This was made possible because of the upper part of the cannula (*11*) which was placed through one of the rubber stoppers. The injecting apparatus was firmly fixed to the socle and the cannula-guide by screws (*12*). By means of this arrangement the solutions were screwed into the brain and very small amounts could be used with satisfactory significance. Another advantage was that different solutions could be used by merely changing the ampulla. As in the electrical stimulations different points could be used for injections by placing pieces of brass (*13*) of different thickness between the injecting apparatus and the socle.

Histological examination:

The animals were sacrificed and the heads treated as in the experiments described above.

Histological examination and localisation of the injection points was facilitated by the injection of 1 % osmic acid at one level. From this point the localisation of the other injecting points was easily determined.

Blood samples were taken before, during and after the experiments and determinations of reducing substances were made according to the method described by FOLIN-WU.

Results:

As for the electrical stimulations a casuistic of some of the injection experiments will be given. The same abbreviations are used to designate side and level of injection. Furthermore when more than one injection at different rostro-caudal levels were made on the same side (in no case more than three) the cannulae are named *A*, *B* and *C*, where *A* is the most anterior one.

Experiment no. 1/53 (Male goat, 9 months).

Blood sugar before the operation was 65 mg % and after 77 mg %. The cannula were inserted on the left side. Three injection levels were used (distance 2 mm). Injection of 0.012 ml., 5 % NaCl at *A. l. u.* resulted in rumination. After 15 minutes the blood sugar value was 64 mg %. Injection of the same amount of NaCl at *B. l. u.* resulted in a very pronounced hyperphagia immediately after the injection. The effect lasted for about 40

minutes. Then the blood sugar value was 59 mg %. Renewed injection at this point resulted in rumination.

Localisation: The point *A. l. u.* (Fig. 11) was situated anterior to the columna fornix descendens near the optic tract. *B. l. u.* (Fig. 12) was situated just in front of the columna fornix descendens.

Experiment no. VI/53 (Lactating goat).

Injections of 5 % NaCl were performed at both sides. The blood sugar value before the operation was about 70 mg %. At *B. l. o.* 0.006 ml. was injected which resulted in hyperphagia.

The same effect was obtained at *B. l. m.* After this injection the blood sugar value was 71 mg %.

After injection at *A. r. m.* (the same amount) hyperphagia was also observed. Blood sugar 67 mg %.

Localisation: B. l. o. was situated between the columna fornix descendens and the mamillo-thalamic tract (Fig. 10) and *B. l. m.* just caudal to the columna fornix descendens (Fig. 11). The localisation of *A. r. m.* was just behind the optic chiasma (Fig. 12).

Experiment no. X/53 (Lactating goat).

Injections with 25 % saccharose were made. Before the experiment the blood sugar value varied between 47 and 49 mg %.

Injection of 0.009 ml. 25 % saccharose resulted in hyperphagia when made at *A. l. m.* After this the blood sugar value was 67 mg %. The hyperphagia lasted about 30 minutes. Injections at other points had no visible effects.

Localisation: A. l. m. was situated just behind the optic chiasma at about the same sagittal section as the columna fornix descendens (Fig. 12).

Experiment no. II/54 (Female goat).

For the injections 3 % NaCl was used. Moderate subdural haemorrhage was observed when burring on the right side.

On injection of 0.006 ml. 3 % NaCl at *C. l. o.* the animal showed pronounced hyperphagia together with slight polydipsia. The same effect except polydipsia was obtained after injection of 0.012 ml. at *C. r. o.*

Localisation: The localisation of the point *C. l. o.* is seen in Fig. 12. The injection was made just lateral to the columna fornix descendens. Point *C. r. o.* (Fig. 11) was situated just anterior to the columna fornix descendens.

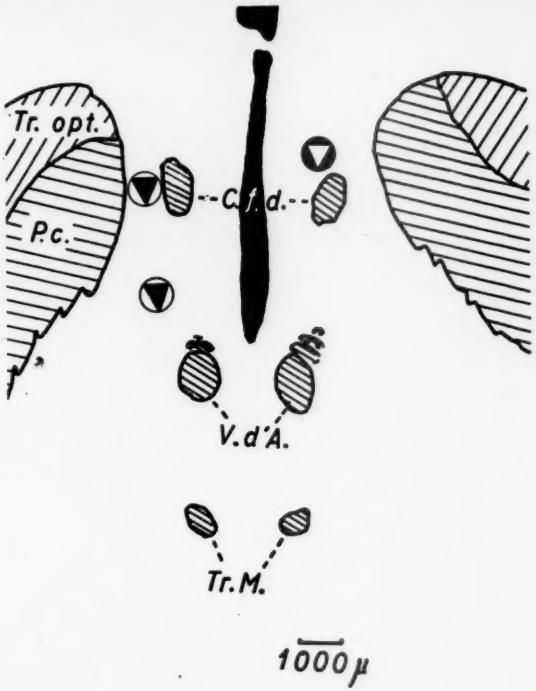

Fig. 10. *Diagram of a horizontal section between the levels o, and m in Fig. 4,* ▼ = Increase of food intake, ▼ = Rumination.
The same abbreviations as in the diagrams above are used.

Experiment no. I/54 (Female goat).

Injections with 3 % NaCl were made. Hyperphagia was observed after injections of 0.009 ml. at *A. l. m.* Injections at *B. l. o.* and *l. m.* also resulted in hyperphagia. At the last point polydipsia was also observed.

Localisation: Figure 11 shows the point *A. l. m.* situated close to the optic tract. The same figure also presents the point *B. l. m.*, localised lateral to the columna fornix descendens. *B. l. o.* seen in Fig. 10 was situated at the border of the cerebral peduncle, lateral to the columna fornix descendens.

Experiment no. V/53 (Lactating goat).

Blood sugar value before the operation was about 70 mg %

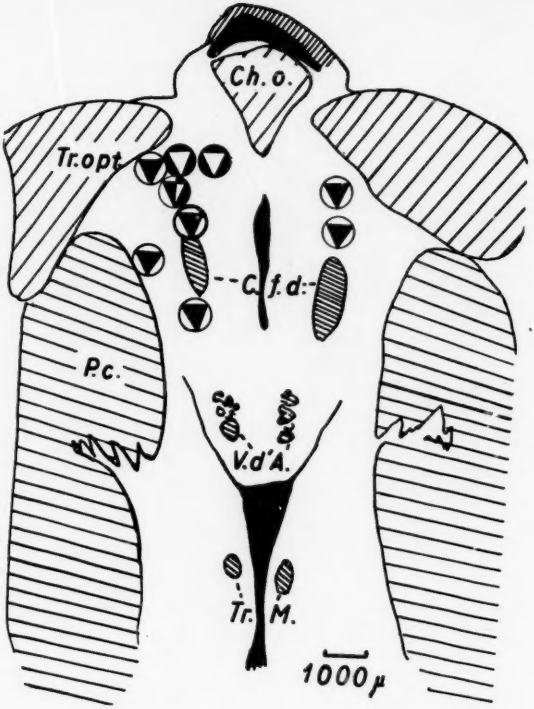

Fig. 11. *Diagram of a horizontal section at level u in Fig. 4.*
▼ = Increase of food intake, ▼ = Rumination. The same abbreviations as in the diagrams above are used.

and after 73 mg %. Injection of 0.006 ml., 5 % NaCl at *A. r. m.* resulted in rumination. When 0.009 ml., 25 % saccharose was injected at *A. l. m.* the animal showed hyperphagia. After this *A. l. m.* produced rumination when 0.009 ml., 25 % saccharose or the same amount 5 % NaCl was injected. The blood sugar value was now 75 mg %.

Localisation: The point *A. r. m.* (Fig. 10) was situated just anterior to the columna fornix descendens and *A. l. m.* (Fig. 11) between the optic tract and the columna fornix descendens. Among isotonic solutions, injected in other experiments, saline, saccharose and glucose were used, and of other substances insulin,

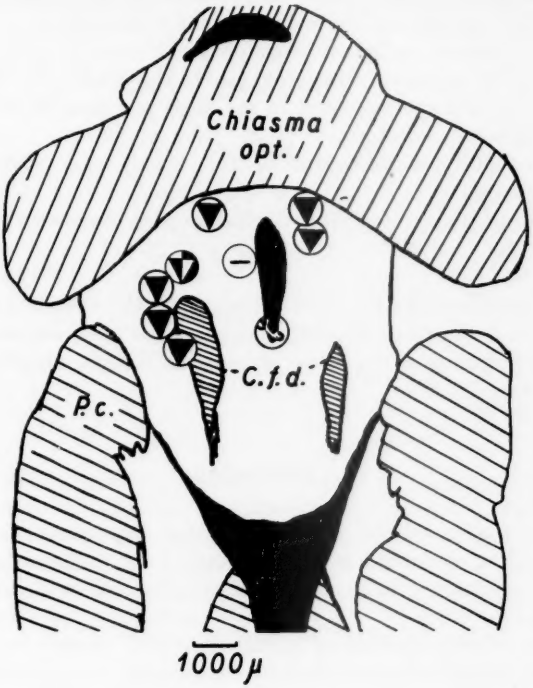

Fig. 12. *Diagram of a horizontal section ventral to level u in Fig. 4.*
▼ = Increase of food intake, ▼ = Rumination, ⊘ = Masticatory and licking movements.
The same abbreviations as in the diagrams above are used.

acetone and ammonia, but no effects of interest for food intake were observed.

A summary of the casuistic shows that:

1. In 16 cases hyperphagia was induced by intrahypothalamic injections of different hypertonic solutions.
2. The histological examination showed that the effective points were situated in a region from the optic chiasma backwards through the hypothalamus, mostly lateral to the columna fornix descendens.

3. The most obvious effects were obtained at the transverse level of, or anterior to, the columna fornix descendens.
4. In 5 cases rumination was obtained mostly where former injection resulted in hyperphagia. In the other cases the effective points were situated in the same region as where hyperphagia was obtained.
5. No significant variations of the blood sugar values were observed.
6. No specificity of different solutions was observed.

Figure 13 shows the results from both the electrical stimulations and the intrahypothalamic injections with respect to the effective points giving hyperphagia, rumination, mastication or licking.

Discussion.

The results related above show that hyperphagia could be induced on sheep and goats by electrical stimulation of different parts of the hypothalamus and the medulla, and by intrahypothalamic injections.

The effective points of stimulation with respect to increase of food intake corresponded to a localisation in the lateral hypothalamus from the optic chiasma backwards through the hypothalamus.

It was observed that the most pronounced effects on food intake seemed to be obtained anterior and lateral to the columna fornix descendens. Here the points of stimulation as a rule corresponded to the localisation of the anterior part of the lateral hypothalamic nucleus or in its vicinity. These results are mostly in accordance to those obtained by BROBECK et al. (1951) who found strong evidence for the existence of a "feeding center" situated in the anterior hypothalamus — in the lateral hypothalamic nucleus. BROBECK et al. found that bilateral destruction of the lateral hypothalamic nucleus in rats was followed by complete absence of eating. If the bilateral lesions were made in the ventro-medial hypothalamic nucleus the animals became obese.

DELGADO and ANAND (1935) later confirmed these findings by demonstrating that an increase of food intake was produced by

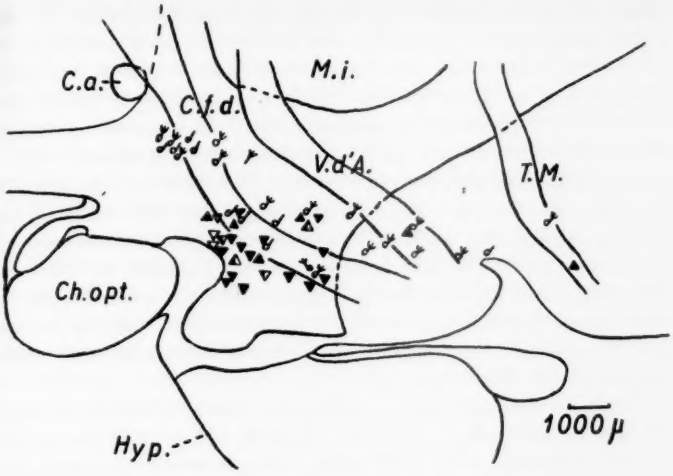

Fig. 13. *Paramedian sagittal section through the hypothalamus.*
▲ = Increase of food intake on electrical stimulation, ▼ = Increase of food intake after injection, ◆ = Rumination on electrical stimulation, ◆ = Rumination after injection, ✓ = Licking movements on electrical stimulation, ✓ = Masticatory movements on electrical stimulation, ✓ = Masticatory and licking movements after injection.
For abbreviations see Fig. 4.

repeated electrical stimulation of the extreme parts of the lateral hypothalamic nucleus. No effect on food intake, however, was observed during the stimulations. The animals received of half a second stimulation (60 cps., pulse duration 0.2 msec., strength 1—5 V) every 5 seconds, for an hour every day, in periods lasting five to ten days. In the experiments described here electrical stimulation of the same parts and intrahypothalamic injections in this region had a very pronounced direct effect on food intake, without simultaneous occurrence of "extra" masticatory and licking movements which was obtained in other parts. This lack of correlation was probably due to different kinds of electrical stimulation or it could be due to stimulation of injection. It is known that different kinds of pulsating current may provoke different effects (Hess, 1948; and Uvnäs, 1947).

Caudal to the columna fornix descendens, but still in the hypothalamus, stimulation resulted in hyperphagia. Brügger

(1943) reported bulimia in cats by electrical stimulation of the hypothalamus and referred to the localisation as the vicinity of the mamillo-thalamic tract at the substantia grisea centralis. BRÜGGER's findings were thus confirmed by these experiments. It was observed, however, that the effect on food intake in this region was often combined with more or less pronounced "extra" masticatory and licking movements. This observation and the fact that hyperphagia was also obtained during stimulation of the anterio-lateral part of the hypothalamus without integration of "extra" masticatory and licking movements might be taken as support for the hypothesis of a higher organisation for the regulation of food intake, presumably in structures related to the anterior part of the lateral hypothalamic nucleus, as previously suggested by BROBECK et al.

From his findings related above, BRÜGGER, suggested a center for the increase of food intake situated in the vicinity of the mamillo-thalamic tract. However, when stimulating regions in the septum pellucidum, dorsal to the anterior commissure, bulimia was also observed. BRÜGGER referred this phenomenon to connections between the structures in the vicinity of the mamillo-thalamic tract and higher centers in the central nervous system. The first center was called the intrinsic, and the latter the extrinsic. According to BROBECK et al., the "feeding center" should be situated in structures corresponding to the localisation of the anterior part of the lateral hypothalamus. In the experiments described here hyperphagia was obtained after stimulation of both "centers", but the most obvious effect was obtained by stimulation in regions corresponding to the anterior part of the lateral hypothalamic nucleus, and it is therefore postulated that this part constitutes an intrinsic locus for an increase of food intake.

It is doubtful if one can talk about centers anatomically separated when dealing with the hypothalamus. The definition of a center fits for other parts of the central nervous system such as the cortex, where almost every function is functionally and anatomically separated. According to HESS (1948) the organisation of a vegetative function in the hypothalamus is never strictly localised to a certain type of cell. This was also found by the present experiments, where the hyperphagia was obtained diffusely but mainly to the lateral hypothalamic nucleus. Because of the simultaneous occurrence of "extra" masticatory and licking movements in the

effect on hyperphagia by stimulation caudal to the columna fornix descendens, a probable explanation is that connections exist between the brain parts regulating food intake and those regulating the "feeding pattern" of the anterior part of the digestive system such as mastication and licking.

The experiments also showed that stimulation (mostly electrical stimulation) of different parts of the hypothalamus resulted in masticatory and licking movements as single effects without concordant occurrence of hyperphagia. Many of these stimulation points were situated in the region where hyperphagia was obtained. MAGOUN, RANSON and FISCHER (1933) found that the substantia nigra was concerned with the masticatory mechanism and HESS et al. (1943 and 1948) showed that rhythmic licking movements could be obtained by electrical stimulation of the ventral thalamic nucleus. Rhythmic licking and mastication was obtained by electrical stimulation of the anterio-lateral part of these structures and the bed nucleus of the stria terminalis. These findings are in accordance with those found in the present experiments. Mastication, licking and swallowing after electrical stimulation were also reported by KAADA (1951).

BABKIN and VAN BUREN (1951) found that electrical stimulation of the cortex resulted in an activation of what they called "feeding pattern". Mastication, inhibition of respiration, salivation, contractions of the esophagus and inhibition of antral motility were typical responses to electrical stimulation of the anterior composite gyrus. However, the most important mechanisms for the regulation of the feeding habit appear to reside in the hypothalamus (ANAND and BROBECK, 1951). STRÖM and UVNÄS (1950) have reported activation as well as inhibition of the motility of the stomach and intestines by electrical stimulation of the hypothalamus.

It thus seems probable that the structures in the hypothalamus regulating food intake also exert influence on the masticatory and licking movements in coordinating these motor functions to be a part of the manifestation of hunger. As mentioned in the review of the literature peripheral sensation plays no predominant rôle for the on-set and regulation of hunger (SHERRINGTON, GROSSMAN and STEIN). This was also supported by the finding of SHARE et al. suggesting an extragastric factor playing a rôle in the regulation of food intake.

In some of the experiments where hyperphagia was observed

this effect was combined with "extra" masticatory and licking movements. These motor effects were often so pronounced that it could be doubtful whether the increase of food intake was primary or not. In such cases it was possible to determine which effect was primary by varying the frequency of the stimulating current.

An obvious hyperphagia was also observed by electrical stimulation caudo-lateral to the mamillary body. This finding might be taken as support for the conception of connections between the hypothalamus and the mesencephalon. MAGOUN (1940) found that descending fibers from the hypothalamus enter the mesencephalon in the medial and lateral bundles. The lateral fibers descend through the lateral hypothalamic area and reach the midbrain by passing dorso-lateral to the mamillary body.

In two of the experiments evidence for connections between the lateral hypothalamus and the medulla, in this case the region of the motor nucleus of the vagus, was obtained. One of the goats (2/53) showed obvious hyperphagia on electrical stimulation lateral and somewhat caudal to the mamillo-thalamic tract. After electro-coagulation at this point nothing special was observed for the first days. After about 7 days the rumen was gradually dilated and the animal showed clinical signs of anterior stenosis (HOFLUND, 1940). The animal was sacrificed 12 days after the coagulation and autopsy verified the clinical diagnosis.[1]

The rumen was greatly enlarged and the stenosis was situated between the reticulum and the rumen. No pathological changes were observed and the stenosis therefore might be referred to the experiment. HOFLUND (1940) showed that anterior stenosis in ruminants very often was due to vagal insufficience. WANG et al. and others found evidence for the existence of vago-hypothalamic connections. It is therefore probable that the stenosis was due to a destruction phenomenon of such connections. It is, however, very curious that such a destruction effect could be provoked because the coagulation was only performed unilaterally.

Hyperphagia was observed on electrical stimulation of the medulla in the vicinity of the dorsal motor nucleus of the vagus. The effect here differed from that obtained by stimulation of the hypothalamus with regard to the animals' choice of food. Stimulation of the medulla produced evident polyphagia. The animals

[1] The clinical diagnosis was confi by Professor S. HOFLUND, Dep. for Cattle and Sheep Diseases, and the opsy (No. P. 746/53) made by Dr. T. NILSSON, Dep. of Pathology, Kungl. Veterinärhögskolan.

showed very pronounced perverse appetite and ate not only food such as hay and straw but also food contaminated with urine and faeces. It might be postulated that certain structures in this part of the medulla constitute some sort of medullary mechanism which, when directly stimulated, presents an undifferentiated ability to increase the food intake without any choice of the kind or quality of the food.

CLARK (1953) studied the nerve control of rumination and reticulo-ruminal motility. He found strong evidence in support of the theory that a subcortical area anterior to the pituitary infundibulum was intimately concerned with rumination and reticulo-ruminal motility.

In some cases in the present experiments stimulation of the hypothalamus resulted in rumination. Mostly the animals became calm and docile and then began to ruminate. This observation was also made by ANDERSSON (1951) who showed that rumination could be obtained after electrical stimulation in structures situated within a region just in front of and lateral to the columna fornix descendens. In the present experiments rumination occurred on stimulation of the regions reported by ANDERSSON, but also caudo-lateral to the columna fornix descendens and in the anterior part of the hypothalamus, where the intrinsic locus for the regulation of food intake is supposed to be situated. In some of the experiments where hyperphagia was obtained, renewed stimulations resulted in rumination. It is not impossible that rumination in this kind of animal constitutes part of the mechanism regulating food intake.

The blood sugar values from the experiments did not show any characteristic changes which could be referred to the effect on the hyperphagia and rumination. These results are in accordance with those obtained by DELGADO and ANAND (1951). In some of the experiments the blood sugar values decreased slowly but in most an increase was observed during the course of the experiments. In all cases an enormous increase was noticed after electro-coagulation, probably due to stress action.

Of the different solutions used for the intrahypothalamic injections no specificity was observed in their action on hyperphagia or the other effects to which attention was paid in these experiments. It appeared that the stimulating effect of the various solutions was due to non-specific osmotic action. Thus mostly only relatively strong hypertonic solutions were active.

Summary.

Electrical stimulation of the hypothalamus and the medulla and intrahypothalamic injections on sheep and goats gave the following results:

1. Stimulation of the hypothalamus just caudal to the optic chiasma backwards throughout the hypothalamus, lateral to a sagittal level through the columna fornix descendens and the mamillo-thalamic tract, resulted in hyperphagia.

2. The most pronounced effect was obtained by stimulation of the region of the lateral hypothalamic nucleus, anterior to the columna fornix descendens or at the same transverse level as this tract.

3. It is suggested that this part constitutes an intrinsic locus for the increase of food intake.

4. The possibility of a relation between the brain parts regulating food intake and the "feeding pattern" is discussed.

5. Polyphagia was observed on electrical stimulation of the region of the dorsal motor nucleus of the vagus.

6. Rumination was obtained on stimulation of the same structures as hyperphagia.

7. No significant changes in the blood sugar values were observed in relation to hyperphagia.

8. The effect obtained by intrahypothalamic injection seemed to be osmotic. No specificity of different substances was observed.

PART II.

Studies of isotope distribution and chemical composition in the hypothalamic region of hungry and fed rats.

By

ARNE FORSSBERG and STIG LARSSON.

In the previous part one of the present authors communicated studies on the sense of hunger in various animals. The finding, that in the hypothalamic region a structure is present which in some way regulates the sense of hunger and which according to MAYER et al. (1952) is the site of so called receptors ("gluco-receptors") encouraged us to the following biochemical approach to the problem. Granted this center has a changed biochemical activity in the brain of hungry rats — either specifically in some biochemical system or as an all over change of the metabolism — tracer studies with radioactive compounds would possibly give some information on the mechanism.

The state of hunger may be expected to cause deviations in the gross distribution of administered isotopes within the body. An increased resorption of activity in a certain organ or tissue may be counterbalanced by a decreased rate in another part. A change in the incorporation rate observed between controls and hungry animals in the particular center under consideration does not necessarily imply anything but a general tendency in common for the brain as a whole. It was essential, therefore, to obtain a versatile conception of the distribution of the injected activity in this particular case. Besides the "hunger center", denoted *C* in this work, two adjacent parts, *A* and *B*, were also analysed at the same time.

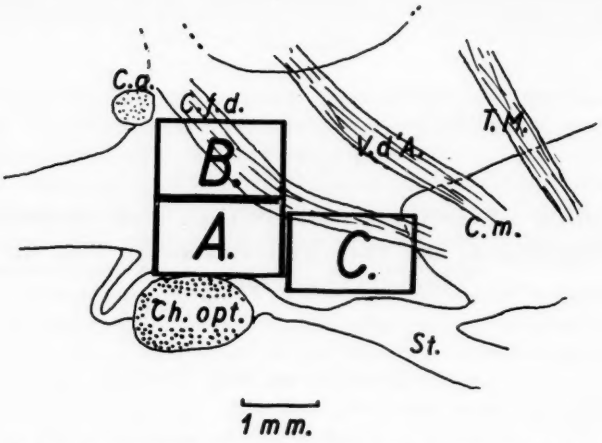

Fig. 14. *Paramedian sagittal section through the hypothalamus of rat.*
A, *B* and *C* = The three samples from the hypothalamus, used for analyses.
C. a. = Anterior commissure; *C. f. d.* = Columna fornix descendens; *Ch. opt.* = Optic chiasma; *C. M.* = Mamillary body; *St.* = Infundibular stalk; *T. M.* = Tractus Meynert; *V. d' A.* = Mamillo-thalamic tract (Tract of Vicq d'Azyr).

The localisation of the three brain parts *A*, *B* and *C* is seen in Fig. 14. Sample *C* is situated in the hypothalamus with exception of its lateral parts including the cerebral peduncles. This part contains the lateral and ventromedial hypothalamic nuclei which together according to BROBECK et al. (1951) constitute the "feeding center". The region of the former nucleus as suggested from the experiments above (p. 36) contained the intrinsic locus for increase of food intake. Samples *A* and *B* were partly situated in the hypothalamic region. *A* included the most anterior parts of the hypothalamus, thus the supraoptic nucleus. *B* included the dorsal parts of the hypothalamus and the ventral parts of the thalamus.

Moreover, samples taken at random from the cerebrum were analysed in a few instances. Total activity per unit volume of blood, liver and muscle was also computed.

This investigation was planned to survey the possibilities in applying the radioactive tracer methods on the present problem. In the first part of the work we proceeded to establish differences

in the resorption rate of isotopes within the hypothalamic region. Following this, attempts were made to arrive at a concept of the reactions involved.

Material and Methods.

Rats. Albino rats from the same strain, male and female, but always of the same sex within each series were used. Age plays a predominant role in accounting for the differential distribution of carbohydrates, especially glycogen, in the brain (CHESLER and HIMWICH, 1943). The animals used were therefore of about the same age (3/4—1 year). Their weight varied somewhat between the different sets of experiment but only slightly ($\pm$ 5 %) within each set. They were fed once a day a standard food up to the start of the experiment.

To ensure that the animals got a physiological hunger they were kept without food for 24 hours only (deprived of one food ration at 10 a.m.). The control animals were kept under ordinary conditions. To ascertain that the animals were hungry 24 hr. later a few animals in each group were given food. They were considered hungry when eating and it was uniformly found that animals not fed for 24 hr. always ate when given food. The control rats showed no or very little interest in food.

Isotope technic. The labelled compounds, diluted by saline, pH $\sim$ 7.0, were injected intraperitoneally in volumes of 0.25 ml. using an Agla Micro syringe. The following compounds were used: $Na_2H^{32}PO_4$, 200 μC/rat; uniformly labelled ^{14}C-glucose, 5 μC/rat and $NaH^{14}CO_3$, 1 μC per rat. The amounts of ^{14}C-labelled compounds available were restricted and did not allow for more than a few experiments. The activities obtained in the small brain samples in ^{14}C-experiments were low and necessitated protracted counting. $Na_2H^{32}PO_4$ was administered as to give a convenient amount for the measurements. All samples to be assayed for activity were pipetted with the syringe from solutions of known concentrations in amounts of 0.2—0.4 ml. on Al-plates 3 cm^2 by area. The Al-plates were covered with thin sheets of linse paper which facilitated a uniform distribution of the solution on the plates. The dried plates (in duplicate if possible) were measured using an end window G-M tube convenient for ^{14}C-measurements.

Owing to the weak radiation emitted from ^{14}C directives for the measurements of "infinite thin layers" were adhered to (KAMEN, 1951). ^{32}P-determinations were always calculated to the day of injection of the isotope. Specific activities were expressed as counts/min. per unit volume. 200 μC ^{32}P is equivalent to 10^8 counts/min. in our device.

Preparation of the samples. To avoid aberrations due to the 24 hours' rhythm in the carbohydrate metabolism the animals in all our experiments were sacrificed at the same hour (10—11 a.m.) in the day. To avoid metabolic changes during this procedure the animals were plunged headlong into liquid oxygen. Further metabolic processes were precluded by keeping the animals in dry air at — 11°C until dissected. The frozen rats were decapitated and the heads were fixed on a bakelite socle by means of carboxygen snow. By careful dissection the skull bones were taken away and the head sectioned transversally by a microtome into sections 20—50 microns thick, using the tape method technic described by PALMGREN (1954). The sections were stained with cresyl violet (B.D.H.) or haematoxylin-eosin and histologically examined for localisation purposes. When the region of the hypothalamus was reached where a sample was to be taken a stamp of known area was pressed down into the brain defining the base area of the sample. Fig. 15 shows the construction of the stamp. The cutting part (*1*) is pressed into the brain with the mandrin (*4*) inserted to avoid rupture in the frozen piece of brain tissue. The thickness of the sample is determined by the stopper (*2*) and the microtome cut. The holder (*3*) is temperature isolated to avoid warming up from the hand. A cube of known volume 3.79 mm^3 $\pm$ 2.5 % was thus obtained. Care was taken to avoid contamination with activity from one sample to the other by cleaning the instruments between each operation. The pieces of substance were immediately transferred to stoppered glass tubes and kept at — 11°C until further analysed.

Chemical fractionations of the three brain samples A, B and C.

For determinations of the total amount of activity in a first set of experiments the frozen pieces were simply hydrolyzed in 0.1-n NaOH and aliquot parts were plated for counting.

In a second set of experiments the brain samples were fractionated by the following procedure giving an acid soluble fraction, a lipid

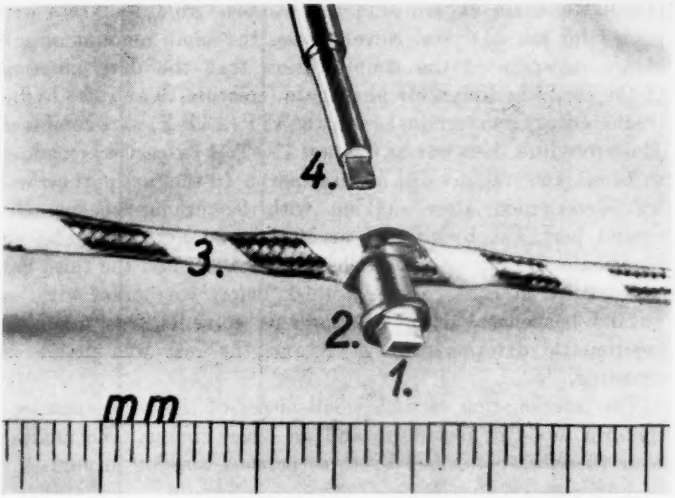

Fig. 15. *The stamp used for preparation of the samples A, B and C.*
1. Cutting part of the stamp.
2. Stopper.
3. Temperature isolated holder.
4. Mandrin.

fraction and a protein-nucleic acid fraction. The frozen pieces were thoroughly homogenized with a glass rod in microcentrifuge tubes, 1.5 ml. by volume, with 0.1 ml. 10 % trichloracetic acid (TCA) working in ice bath. The TCA volume was made up to 0.7 ml. and extraction continued for 5 min. The samples were spun down in a chilled microcentrifuge at 10,000 RPM, the solvent was thoroughly separated and the procedure repeated with 0.7 ml. 5 % TCA. The residue was washed with 0.7 ml. ice cold distilled water. The lipids were then extracted twice with 0.7 ml. alcohol-ether 3 : 1 at 50°. The residue after lipid extraction, comprising the nucleic acids and proteins, was dried and hydrolyzed in 0.1-n NaOH.

All the solvents obtained were made up to known volumes and aliquot amounts were plated for activity measurements. Due to the small amounts of activity present, especially in the lipid fraction it was necessary in these experiments to pool the samples from two or three rats before analyses.

In a third set of experiments the acid soluble (TCA) fraction was further analysed following the procedure of ERNSTER et al.

(1950). In these experiments the samples from four rats were pooled for the analyses. Nevertheless, the small amount of substance necessitated the simplification that the determinations of the easily hydrolysable phosphate (creatine-P) and the hydrolysable energy rich terminal groups of ATP (ATP-P) were combined. The procedure then was as follows. The TCA extract was made up to 10 ml. and was divided in three parts. In the first part ortho-P was determined after shaking with isobutanol-benzene. The second part was hydrolyzed at 100° in 20 min., releasing the phosphate from creatinephosphate and ATP, and the third part was burned in H_2SO_4 giving total-P before extraction with isobutanol-benzene. Part of the organic solvents was taken for colorimetric determination of P and the rest was plated for counting.

The fractionation of such small pieces of tissue necessitates a uniform mode of procedure and an exact timing. The analyses were therefore performed by three persons working in parallel.

Results.

Resorption of ^{32}P in the unfractioned hypothalamic samples and in some other tissues. It would seem that ^{32}P might be a convenient tracer for a survey of the isotope uptake in the brain samples as the number of phosphorylating processes are numerous and the brain is rich in phosphorus. The main part of the investigation was also performed using $Na_2H^{32}PO_4$.

The rate of resorption of ^{32}P into the three parts of the brain considered in this work was not known. It is known, however, that the resorption of intraperitoneally injected phosphorus in the brain as a whole is a rather slow process (Hevesy, 1939; Hahn and Hevesy, 1940). On the other hand ^{32}P rapidly enters into metabolism when resorbed in the tissue.

In the first trial the animals were administered the isotope at the end of the 24 hr.-fasting period. They were sacrificed in groups of four (two hungry and two fed) 15, 30 and 60 min. after the injection. The analyses showed the general tendency indicated in Table 1, containing the whole material. Variations being rather pronounced in the 15—30 min. groups, repeated experiments were mainly performed at 60 min. isotope time. In several sets of experiments the isotope was injected simultaneously with the

Table 1.

Resorption of ^{32}P in the unfractioned hypothalamic samples A, B and C at various times after injection of the isotope.

Isotope time	Samples analysed	Hungry					Fed					Significance test for the diff. hungry : fed			
		Counts/min/mm³ of:			Ratio of:		Counts/min/mm³ of:			Ratio of:		C/A		C/B	
		A	B	C	C/A	C/B	A	B	C	C/A	C/B	t	P	t	P
15 min.	4	**25** ± 8.5	**16** ± 2.0	**35** ± 5.2	**1.40** ± 0.519	**2.19** ± 0.426	**45** ± 2.2	**24** ± 0.88	**40** ± 7.6	**0.889** ± 0.174	**1.67** ± 0.323				
30 min.	10	**29** ± 8.8	**10** ± 2.4	**49** ± 15.9	**1.69** ± 0.751	**4.90** ± 1.98	**54** ± 15.4	**28** ± 6.0	**44** ± 17.2	**0.851** ± 0.394	**1.57** ± 0.701	1.03	< 0.4	0.972	< 0.4
60 min.	22	**52.2** ± 5.40	**30.7** ± 3.56	**75.6** ± 5.15	**1.67** ± 0.299	**2.89** ± 0.417	**72.0** ± 18.9	**39.5** ± 4.78	**40.0** ± 3.84	**0.763** ± 0.125	**1.14** ± 0.173	2.81	~ 0.01	3.88	< 0.001
24 hr.	22	**125.5** ± 7.05	**105.8** ± 6.25	**186.6** ± 15.1	**1.49** ± 0.103	**1.77** ± 0.121	**132.4** ± 5.65	**107.6** ± 4.60	**132.1** ± 9.40	**0.986** ± 0.0942	**1.20** ± 0.0878	3.60	~ 0.001	3.79	~ 0.001

start of the fasting period. These experiments thus gave us the net results of the ^{32}P turn-over during the development of the hunger. An inspection of the figures for ^{32}P-activity per mm^3 shows that the activity of *C*-samples from hungry animals is high compared with the corresponding values for fed animals. Conversely, the *A* and *B* samples of hungry animals contain less activity than do fed ones. Thus, an unequal distribution of ^{32}P occurs within this small volume of adjacent samples.

This mode of distribution led us to consider a formation of ratios between the activity values as being probably the best quantitative expression for the differences between hungry and fed animals. The procedure can be accomplished in different ways.

We have preferred the representation indicated from the Table 1, because a comparison between the uptake of activity in the three adjacent brain samples of the rats takes into account the characteristic pattern of an uneven distribution of ^{32}P within the whole hypothalamic region. The biological variations between the single rats caused differences in the absolute amount of ^{32}P reaching the hypothalamus. As a consequence the mean values for the activities in *A*, *B* and *C* are impaired by rather great errors, as are the mean ratios which can be calculated from these mean values. It appears, that a more correct estimate of the measure *C*/*A* and *C*/*B* is arrived at when the ratios of the activity determinations on each single rat is first computed, followed by a calculation of the mean ratios. It may be noted that the figures obtained from this mode of computing the mean ratios differs somewhat, but not significantly, from the corresponding figures calculated from the means of *A*, *B* and *C* in the table. The mean ratios for *C*/*A* and *C*/*B* being formed within each group of animals, a statistical test for the significance of the differences between hungry and fed was applied.

In hungry rats the sample *C* which constitutes "the feeding center" shows a preferential uptake of ^{32}P indicating an increased physiological activity over that in the fed state. Although the experimental material at 15—30 min. is limited it would seem that the tendency is manifested shortly after the injection of the isotope. Injections concomitant with the start of the hunger period also gave consistent results (*vide* 24 hr. exp.).

When the activity per unit volume in the three samples *A*, *B* and *C* in each group of animals is summed up it appears that the

differences between hungry and fed animals are rather small and in any case much smaller than the differences between the single fractions *A*, *B* and *C*. From Table 1 the following figures for the relative resorption rate in $\frac{\text{hungry}}{\text{fed}}$ can be computed: at 15 and 30 min. = 0.7, at 60 min. = 1.05 and at 24 hr. = 1.12. Similarly, the integrated values from Table 4 give the ratios 1.17 and 1.12 at 60 min. respectively 24 hr. Possibly the total activity in the hypothalamic region of hungry rats is on an average slightly higher (~ 10 %) from 60 min. on. This fact suggests that the distribution of ^{32}P to the whole hypothalamus is not appreciably impaired by the hunger. The mechanism responsible for the uneven partition of the activity must then be sought within the hypothalamus.

Table 2.

^{32}P-activity in various organs; average of determinations at 15 min.—24 hr.[1]

Organ	Group	Counts/min./mm³	Significance test for the difference hungry : fed	
			t	P
Blood	Hungry	312 ± 38.6	0.423	0.6—0.7
»	Fed	292 ± 27.2		
Liver	Hungry	1793 ± 130.0	3.98	< 0.001
»	Fed	1264 ± 27.9		
Muscle	Hungry	259 ± 24.2	0.476	0.6—0.7
»	Fed	242 ± 26.2		
Cerebrum	Hungry	119 ± 19.0	0.330	0.7—0.8
»	Fed	127 ± 15.3		

As stated above an inequality in the partition of the injected isotope may be expected when comparing the uptake of activity into various tissues of hungry and fed rats. It appears from Table 2 that the partition in a number of tissues shows a close agreement between the two groups of animals. In particular, the fact that the cerebrum samples, show a similar uptake of ^{32}P in both groups emphasizes furthermore the specificity of the differential incorporation rate found by us in the hypothalamus. It is also of interest to note that the blood activity did not differ

[1] No principal differences due to variations in isotope time were observed.

in the two groups. The liver from hungry animals, however, incorporates significantly more ^{32}P (42 % on an average) than does the liver from normal ones. As the amount of activity in the liver is very high one should expect the surplus activity in liver from hungry animals to be counterbalanced by a deficit in other parts of the body. We have not considered a complete survey of the distribution of the activity; the figures given may suffice to show some features of importance for a judgement of the differences in the brain parts especially considered in this work.

Total-P determinations.

Although very unlikely, the possibility that the hunger causes changes in the total phosphorus concentration could not be overlooked. Determinations of total phosphorus (Table 3) did not lend support to this concept. Probably, however, the phosphorus content in the sample *B* is smaller which would partly account for the low radioactivity values. An alternative explanation for the latter fact would be that the sample *B* is situated in a region with a poorer blood supply than the other samples.

Table 3.

Total phosphorus: γ P per mm^3 of brain substance in samples A, B and C.

Hungry			Fed		
A	B	C	A	B	C
2.74 ± 0.33	2.14 ± 0.38	2.38 ± 0.31	2.32 ± 0.31	2.13 ± 0.12	2.33 ± 0.39

The distribution of activity on the acid soluble fraction, the lipid fraction and the protein-nucleic acid fraction.

The proceeding results having established that hungry and fed animals differ in respect of their incorporation of ^{32}P into the three hypothalamic samples, it seemed logical to fractionate the samples in the way indicated. These experiments were performed on rats which had been given the isotope 60 min. respectively 24 hr. before sacrificing (Table 4).

After 60 min. the isotope is incorporated into the three fractions with preference for the acid soluble compounds. Lipid

Table 4.

Incorporation of ^{32}P into TCA, lipid and protein + nucleic acid (NA) fractions of the samples A, B and C; 60 min.

Table 4.

Incorporation of ^{32}P into TCA, lipid and protein + nucleic acid (NA) fractions of the samples A, B and C; 60 min. (I) respectively 24 hr. (II) after injection of isotope.

Fractions	Samples analysed	Hungry					Fed					Significance test for the diff. hungry : fed			
		Counts/min/mm³ of:			Ratio of:		Counts/min/mm³ of:			Ratio of:		C/A		C/B	
		A	B	C	C/A	C/B	A	B	C	C/A	C/B	t	P	t	P
I.															
TCA	6	55.3 ± 4.64	36.0 ± 2.69	68.3 ± 4.55	1.25 ± 0.0642	1.95 ± 0.137	50.5 ± 8.45	44.2 ± 5.19	40.6 ± 4.0	0.948 ± 0.171	1.02 ± 0.179	1.68	< 0.2	4.12	≃ 0.005
Lipids	6	1.33 ± 0.26	0.62 ± 0.13	1.71 ± 0.34	1.54 ± 0.384	3.90 ± 1.35	1.31 ± 0.37	1.53 ± 0.33	1.33 ± 0.42	1.17 ± 0.320	1.03 ± 0.357	0.765	0.4–0.5	2.96	< 0.02
Proteins-NA's	6	4.69 ± 0.52	3.22 ± 0.53	6.09 ± 0.58	1.32 ± 0.070	2.07 ± 0.102	3.64 ± 0.62	3.77 ± 0.60	3.63 ± 0.82	1.19 ± 0.196	1.10 ± 0.265	0.608	0.5–0.6	2.33	< 0.05
II.															
TCA	4	81.4	62.5	76.7	0.954	1.23	87.0	63.4	50.0	0.558	0.790				
Lipids	4	20.9	18.2	28.4	1.28	1.61	24.5	18.9	21.2	0.884	1.12				
Proteins-NA's	4	14.5	12.8	17.5	1.22	1.38	14.7	12.8	13.2	0.903	0.947				

activity is very low at 60 min., but increases rapidly, giving higher values than the proteins in the 24 hr.-experiments. Our figures suggest that at 60 min. 90 per cent of the total activity is present in the TCA-fraction against only 2—3 per cent in the lipids and 7—8 per cent in the proteins-nucleic acids. After 24 hr. the corresponding figures were: TCA-fraction 65 per cent, lipids 20 per cent and proteins-nucleic acids 15 per cent. Our figures for 24 hr. agree rather well with those of DZIEWIATKOWSKI and BODIAN (1950) who found on an average 60, 30 and 10 per cent ^{32}P respectively in the mentioned fractions. These values were obtained from analyses of the whole brain of rats.

It appears from Table 4 that the unequality in the distribution of activity between the samples *A*, *B* and *C* is rather uniformly manifested in all the three fractions. There seems to be no definite preference in the incorporation rate for any fraction as indicated from the ratios *C*/*A* and *C*/*B*. The ratio *C*/*B* is very high for the lipids of hungry rats at 60 min., but the determinations after 24 hr. give the impression of almost equal differences in the ratios of hungry and fed animals. It may be noted that the determinations at 24 hr. show an all over decrease in the ratios of both hungry and fed rats. This may be due to a rapid release of ^{32}P from tissues showing a high resorption shortly after administration of the isotope.

The TCA-fraction. This fraction, containing low molecular compounds and intermediates in the carbohydrate metabolism, among others ATP-ADP, creatinephosphate, hexosephosphates and glycerophosphates besides the orthophosphate, is of special interest because a study of the distribution of activity herein may be informative concerning the primary chemical reactions. In particular ATP holds a central position in the energy cycle of the cell.

Already 15—30 min. after injection of the isotope the familiar pattern of ^{32}P-distribution was manifested (Table 1). One should not expect biochemical reactions in which ^{32}P participates to have proceeded to any considerable extent at that time. It is likely, therefore, that the main bulk of activity is still present as ortho-P. A single experiment, sacrificing the rats 30 min. after the injection, confirmed this assumption. Rather low activity values were found in all other fractions except ortho-P. With regard to the latter fraction the ^{32}P-distribution on samples

of hungry and fed rats agreed with the principle outlined in Table 1. This suggests that the unequal ortho-^{32}P-distribution on the three samples of hungry and fed rats occurs shortly after injection of the isotope.

The three main experiments were performed at 24 hr., pooling the samples from four rats in each instance. Essentially the analyses established that very marked changes occur between the samples in regard to the concentration of ATP-P + creatine-P whereas the concentration of ortho-P was found to vary less. These analyses which seems us to have an important bearing on the problem of the functional mechanism are given in Table 5 A.

It is of interest to note that the concentration of the combined organic fractions and of ortho-P in the three added samples $A + B + C$ does not differ appreciably between hungry and fed as ascertained by the ratios which are close to 1. This agrees with the corresponding results of ^{32}P-determinations (p. 49). Here, again, a mechanism is indicated by way of which the biochemical activity within the hypothalamic region is autonomously regulated. The mutual partition of ortho-P between the samples shows somewhat higher ratios in hungry rats, particularly when comparing the samples *C* and *A*, but the differences are, when compared with those in the ATP-P + creatine-P fraction rather small. The latter fraction shows marked variations in respect of the mutual distribution between *C* and *B*. The differences are of a similar nature as the general ^{32}P-distribution, although quantitatively still more pronounced. It appears that each single determination (which comprises the combined samples from four rats) of the *C*-sample from hungry rats are much higher than the corresponding *B*-sample, while the opposite relationship is valid in fed rats. A test for the significance that such a difference would occur by random distribution gives 1 : 64. However, this is certainly an underestimation of the significance for the difference hungry : fed as it does not take into account the quantitatively great differences, giving on an average a 6 times higher ratio in hungry rats.

The behaviour of the *A*-samples differs from the general scheme; it seems as if the ratio C/A is slightly higher in fed rats than in hungry. The concentration of ATP-P + creatine-P in *A*-samples is markedly low in fed rats combined with rather high ortho-P values.

Table 5 A.

Concentration of ortho-P and ATP-P + creatine-P expressed as γ P $\times$ 10^{-2} per mm^3 of tissue.

Experiment	Hungry										Fed									
	ortho-P					ATP-P + creatine-P					ortho-P					ATP-P + creatine-P				
	A	B	C	C/B	C/A	A	B	C	C/B	C/A	A	B	C	C/B	C/A	A	B	C	C/B	C/A
1	45	46	43	0.94	0.96	10.5	7.5	10.5	1.40	1.0	50	41	40	0.98	0.80	7.5	17.4	10	0.58	1.33
2	59	59	51	0.87	0.87	—	5.0	19.6	3.92	—	68	64	49	0.77	0.72	5.0	14.7	7.3	0.50	1.46
3	54	46	51	1.11	0.95	12.3	2.6	12.3	4.73	1.0	73	64	51	0.80	0.70	5.0	12.0	7.3	0.61	1.46
Mean	52.7	50.4	48.4	0.97	0.93	11.4	5.03	14.1	3.36	1.0	63.7	56.4	46.7	0.85	0.74	5.84	14.7	8.2	0.56	1.42
Σ	151.5					30.5					166.8					28.7				

Table 5 B.

^{32}P-activity, calculated on the total amount of each fraction present per mm^3 of tissue.

Hungry										Fed									
ortho-^{32}P					ATP-^{32}P+creatine-^{32}P					ortho-^{32}P					ATP-^{32}P+creatine-^{32}P				
counts/min/mm^3 of:			Ratio of:		counts/min/mm^3 of:			Ratio of:		counts/min/mm^3 of:			Ratio of:		counts/min/mm^3 of:			Ratio of:	
A	B	C	C/B	C/A	A	B	C	C/B	C/A	A	B	C	C/B	C/A	A	B	C	C/B	C/A
25.1	20.7	27.3	1.32	1.08	0.451	0.306	0.588	1.93	1.30	32.1	27.1	21.3	0.79	0.66	0.392	1.50	0.379	0.25	0.97
Σ 73.1					1.345					80.5					2.273				

To conclude; the ATP-P + creatine-P fraction shows the same mode of distribution between *C* and *B* samples as was found for ^{32}P, while the ortho-P, being the pool of inorganic phosphate available for organic synthesis, does not vary appreciably.

The corresponding values for the ^{32}P-activity, calculated on the total amount of each fraction present per mm^3 of tissue (Table 5 B), ascertain that ^{32}P has been distributed giving high ratios for C/B in hungry rats compared with low ratios in fed ones. In particular the difference hungry : fed is very marked in the ATP-P + creatine-P fraction. This is of course a consequence of the differences in the total amount of the fraction. It may be noticed that the activity determinations display the usual difference in the C/A ratio.

Experiments with ^{14}C-Glucose. The amount of ^{14}C-glucose available did not allow for more than a test on four rats (two hungry and two fed). The results are therefore given but to show the tendency. From the injected activity the brain parts took up ^{14}C 45 min. after injection of the isotope in amounts corresponding to 8—15 counts per mm^3. The ratios were found to be: for fed rats $C/A = 1.10$ and $C/B = 1.06$. For hungry rats the corresponding figures were $C/A = 1.29$ and $C/B = 1.18$. In these experiments, again, the liver of the hungry rats incorporated more activity (45 %) while the blood and muscle uptake was slightly higher in the fed rats. The differences are small but suggest a similar mode of distribution as was found in the ^{32}P-experiments.

Experiments with $NaH^{14}CO_3$. The aim of these experiments was to study the distribution, using a labelled compound which does not take part in the metabolism proper. By far the greatest amount of the injected $NaH^{14}CO_3$ is exhaled as $^{14}CO_2$ at a very rapid rate. Forssberg and Hevesy (1954) found 10—15 per cent to be exhaled by mice in the first 2 min. and about 65 per cent in the first 10 min. after an intraperitoneal injection. The $H^{14}CO_3'$, left in the body, being a component of the buffer system, should be expected to appear mainly in inorganic form at short intervals after injection.

The rats (6 hungry and 6 fed) were injected with sodium bicarbonate at the end of the fasting period and sacrificed from 3 to 25 min. after the injection which took 15 sec. About 11 min. after

the injection of the isotope low activities could be ascertained in the brain samples. Because of the long times necessary for counting only the C and B fractions were measured. The familiar pattern appeared already 11 min. after injection. We therefore pooled the measurements from all samples taken between 11 and 25 min. (from 8 rats in total) and obtained: for hungry rats, $C/B = 1.08 \pm 0.209$, and for fed ones $C/B = 0.78 \pm 0.182$. The differences are, again, not so pronounced but agree with the ^{32}P results.

Discussion.

In the present part of the work we were mainly concerned with a demonstration of the basic fact that biochemical differences occur in the hypothalamic region. Using ^{32}P as a tracer it was shown that the activity is incorporated in the three brain samples within this region according to a characteristic pattern which distinguishes hungry rats from fed ones. The results obtained with ^{32}P were amplified in experiments with ^{14}C-glucose and $NaH^{14}CO_3$.

According to HIMWICH (1951) the brain should not be regarded as an entity; rather its specialised parts are distinguished by a chemical activity of their own. The investigations of BORELL and ÖRSTRÖM (1945) corroborated this view. These authors are of the opinion that the differences in the uptake of ^{32}P in various brain parts may be ascribed to differences in the biochemical activity. With respect to the mechanism other views are also expressed. BRATTGÅRD and LINDQVIST (1954), using ^{82}Br were inclined to believe that the resorption of isotopes is regulated through interaction of "brain barriers".

When surveying our material from the point of view of the mechanism involved it seems us of particular interest to stress the following facts concerning the gross distribution of ^{32}P in the body. The activity values of the blood (taken from the heart) did not vary significantly between the two groups. Thus, one may assume that the supply to the brain, taken as a whole, is similar, too. This fact has also been stated by several workers (KERR and GHANTUS, 1936; and others). The determinations on samples from the cerebrum are in line with the blood values. No "brain barrier"-differences between hungry and fed rats are, therefore, operative in the cerebrum.

There is also evidence for the assumption that the total activity within the hypothalamus of hungry and fed rats is equal. This is supported by the fact that the integrated activity from the samples *A*, *B* and *C* (cp. Fig. 14) was found to be similar (Tables 1 and 4). We arrive then at the conclusion that the mechanism responsible for the unequality of ^{32}P-distribution is localised within the hypothalamic region as no "brain barrier"-differences are operative in this instance either.

The question then arises as to the nature of the forces giving rise to the unequal distribution of isotopes in *A*, *B* and *C*. It is of interest to note in this connection that the determinations of hydrolysable terminal groups of ATP and creatine-P indicate a concentration of these compounds in the three samples which mainly coincides with that of the activity distribution. Considering the rather uniform distribution of ortho-P, being the pool of inorganic phosphate available for synthesis of organic P, the marked unequality in the concentration of the mentioned organic fraction is particularly noteworthy.

Furthermore, the concentration of the organic compounds in the whole hypothalamic region does not differ very much in hungry and fed rats. Thus, apparently the same principle found in the distribution of total-^{32}P on the whole unfractioned samples as well as in the distribution on the TCA, lipid and protein-nucleic acid fractions is again met with in the ATP + creatine-P fraction.

HESS (1923) postulated a differentiated blood supply directly related to the cerebral activity. NACHMANSOHN and MACHADO (1943) have shown that homogenized brain tissue synthezises acetylcholine (Ach) in the presence of ATP. The latter compound should according to these authors be capable of delivering energy for the acetylation of choline. In this manner ATP plays an important role in the metabolic cycle of nerve cells (NACHMANSOHN, 1952). An increase of Ach in the tissue would be expected to cause a local capillary dilatation in regions of high functional activity as being postulated for sample *C* in hungry rats. In general, one should expect the ATP-concentration to determine the blood supply and consequently also the amount of ^{32}P available in the different hypothalamic samples. According to this line of thought the resorption of the radioactive isotopes is best understood as a logical sequence in the suggested schedule. In particular, the pattern of ^{32}P-distribution found in the lipids and proteins-nucleic acids, which is otherwise difficult to understand, follows as a conse-

quence of the unequal ortho-^{32}P-distribution. It may be noted that besides Ach, certain breakdown products of ATP (AMP and adenosine) also act as vasodilators (DRURY, 1936). This suggests further possible explanations for the effects found in our work.

Although the increased resorption of ortho-^{32}P in the *C*-sample of hungry rats would, apart from other factors, account for the high ^{32}P levels in the lipids and proteins-nucleic acids it must be borne in mind that a high ATP-concentration also directly should be expected to cause an increased biochemical activity in general. It is not easy, therefore, to judge whether the variations in ortho-^{32}P uptake completely accounts for the degree of incorporation of ^{32}P in the mentioned high molecular compounds or whether also variations in their biochemical activity do occur.

Obviously the hunger state does not cause changes merely in the "feeding center", *C*. Rather the impression is obtained of a closely correlated chain of reactions in which various parts of the hypothalamic region participate. There seems to be a shift in the ATP-concentration from one part of the hypothalamus to another and consequently also a shift in the energy metabolism. Although no detailed analyses are so far available one should be inclined to believe that the reversible reaction ATP $\rightleftharpoons$ ADP plays a predominant role in this chain.

The physiological interpretation of this observation can be related to HESS's (1948) conception of the hypothalamus as the superior regulating region for "trophotrop-endophylaktische" and "ergotrop-dynamogene" functions. According to this theory stimulation of structures related to these functions should result in a state, corresponding respectively to parasympathetic and sympathetic activity.

Summary.

1. The aim of this investigation was primarily to ascertain whether any differences in the resorption occur within the hypothalamic region from hungry (*H*) and fed (*F*) rats.

2. The distribution of isotope labelled radioactive compounds, preferably $Na_2H^{32}PO_4$, but also ^{14}C-glucose and $NaH^{14}CO_3$ was studied in three samples within the hypothalamic region of *H* and *F* rats. One of these samples, denoted *C*, contained the

"feeding center" regulating food intake. The other, adjacent, samples *A* and *B* were analysed for comparison as were also samples from blood, liver, muscle and samples taken at random from the cerebrum.

3. The sample *C* resorbs significantly more ^{32}P in *H* than in *F* rats. Simultaneously, the two samples *A* and *B* were less active in *H* than in *F* rats. This pattern of distribution is expressed through a formation of ratios for the activity found in *C*/*B* and *C*/*A* within each group of rats.

4. The total activity incorporated in these three samples taken together was similar in the both groups of rats; thus only the mutual distribution within the hypothalamic region differs between *H* and *F*.

5. Neither the activity of the blood nor of samples taken at random from the cerebrum showed any significant differences between *H* and *F* rats. That the blood activity is similar in *F* and *H* suggest that the ^{32}P flow through the brain is also similar; this conclusion is also indicated from the analyses of the cerebrum. This fact emphasizes the specificity in the behaviour of the hypothalamic region.

6. The concentration of ATP + creatinephosphate was markedly higher in *C*-samples than in *B*-samples of *H* rats. The opposite relationship was found in *F* rats. The high concentration in *C*-samples of *H* rats may be related to an increase of Ach, followed by a local capillary dilatation. This would cause an increased resorption of the tracers.

7. Following the increased uptake of ortho-^{32}P, which was ascertained shortly after the ^{32}P-injection, a corresponding rise in the activity of some biochemical systems *viz.* lipids, proteins and nucleic acids was found. Conversely, in samples with low ATP + creatine-P-concentration also the ^{32}P activities were low.

General Conclusions.

In the first part of this investigation induced hyperphagia was studied in sheep and goats. This corroborated the existence of hypothalamic structures regulating food intake. An activation of some of the oral "feeding pattern" such as mastication, licking and rumination was also observed.

In the second part the biochemical mechanism was studied in rats under a state of physiological hunger. The results of these experiments suggested that changes in the concentration of ATP + creatine-P in the hypothalamic region in question constitute the driving force for the postulated higher functional activity. It was shown that an increased resorption of injected isotopes occurs in this part, probably related to a local capillary dilatation.

It is likely that the hypothalamic structures found to produce hyperphagia in sheep and goats also possess a higher functional activity which is regulated through a similar mechanism as in the state of natural hunger.

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